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(54) An automatic synthesizer for DNA

(57) An automatic synthesizer is for synthesizing DNA or the like with solutions of a plurality of reagents being fed to its reactor. The reactor consists of a container having at the top thereof an inlet for reagent solutions, a filter for synthesis of DNA or the like set inside thereof at a lower level on which a charge of solid support for synthesis of DNA or the like can be placed and which is permeable to any reagent solution and a liquid outlet in the bottom thereof and also having a reaction space of a minute volume above the filter. The reactor is further provided with a reagent solution feed means capable of feeding solutions of reagents in quantities according to the charged quantity of the solid support.

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Certain of the Chemical formulae appearing in the printed specification were submitted after the date of filing, the formulae originally submitted being incapable of being satisfactorily reproduced.
The drawings originally filed were informal and the print here reproduced is taken from a later filed formal copy.

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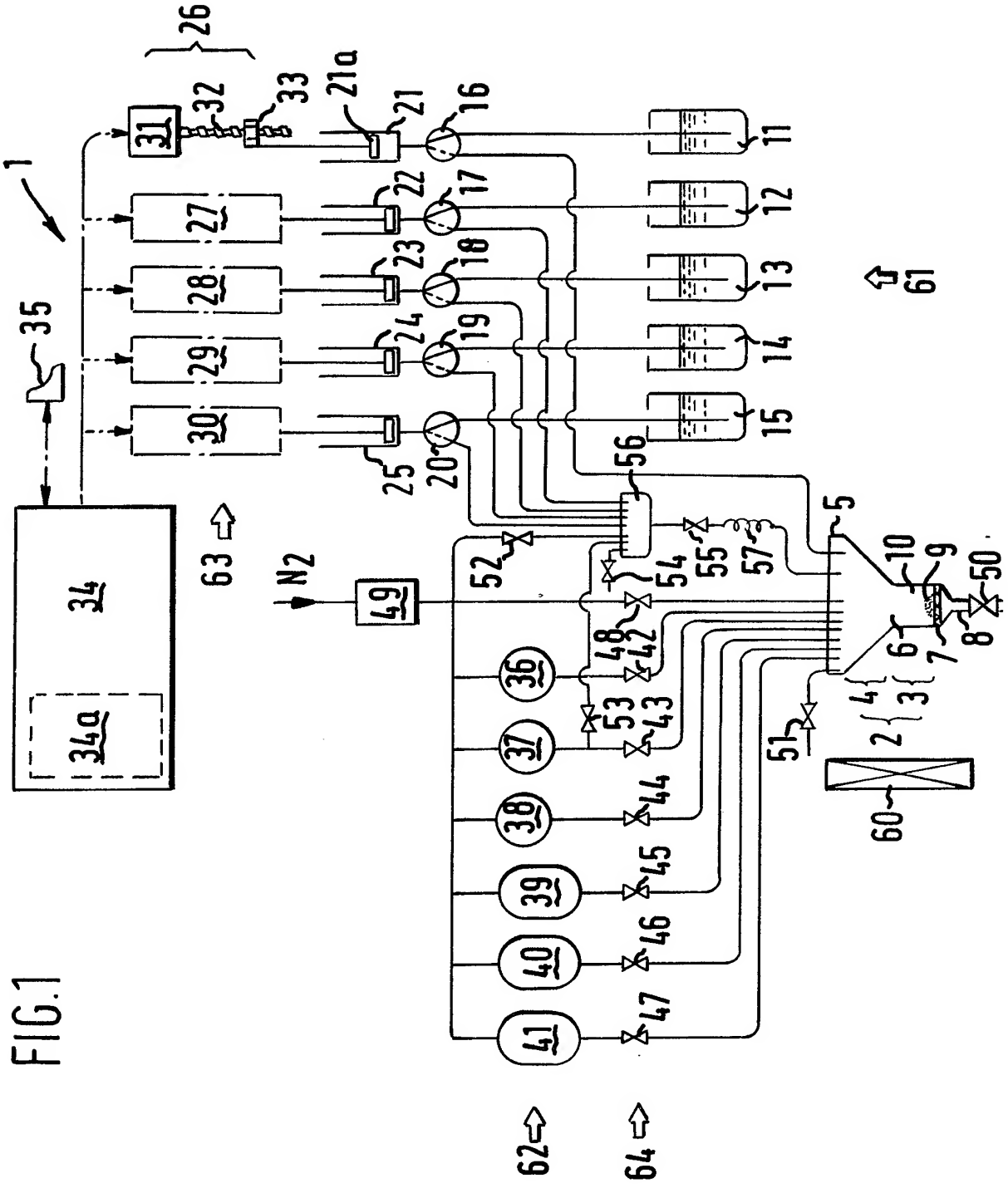


FIG.1

FIG.2

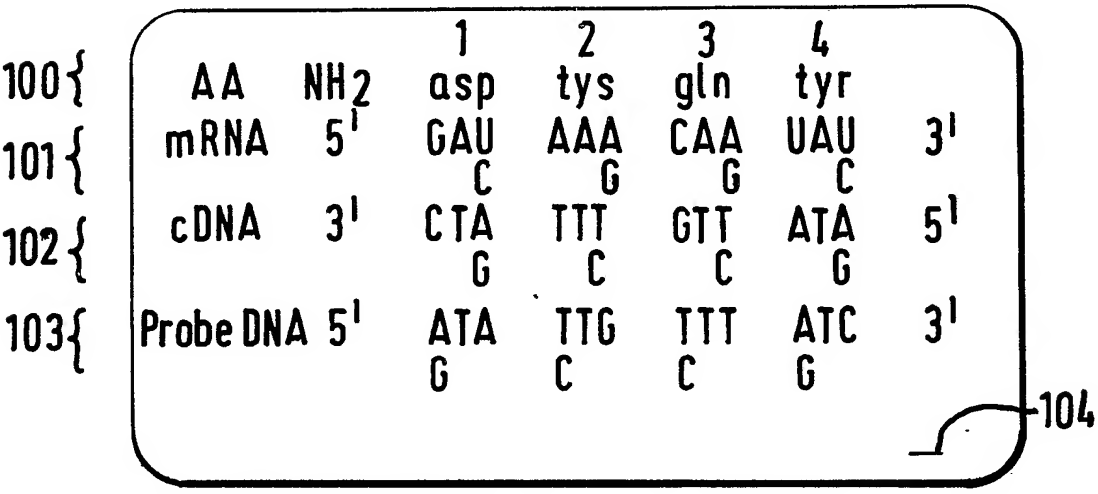
2nd. character U / 1st. character	3rd. character U	C	A	G
U	phe	phe	leu	leu
C	leu	leu	leu	leu
A	ileu	ileu	ileu	met
G	val	val	val	val

2nd. character C / 1st. character	3rd. character U	C	A	G
U	ser	ser	ser	ser
C	pro	pro	pro	pro
A	thr	thr	thr	thr
G	ala	ala	ala	ala

2nd. character A / 1st. character	3rd. character U	C	A	G
U	tyr	tyr		
C	his	his	gln	gln
A	asn	asn	lys	lys
G	asp	asp	glu	glu

2nd. character G / 1st. character	3rd. character U	C	A	G
U	cys	cys		trp
C	arg	arg	arg	arg
A	ser	ser	arg	arg
G	gly	gly	gly	gly

FIG.3



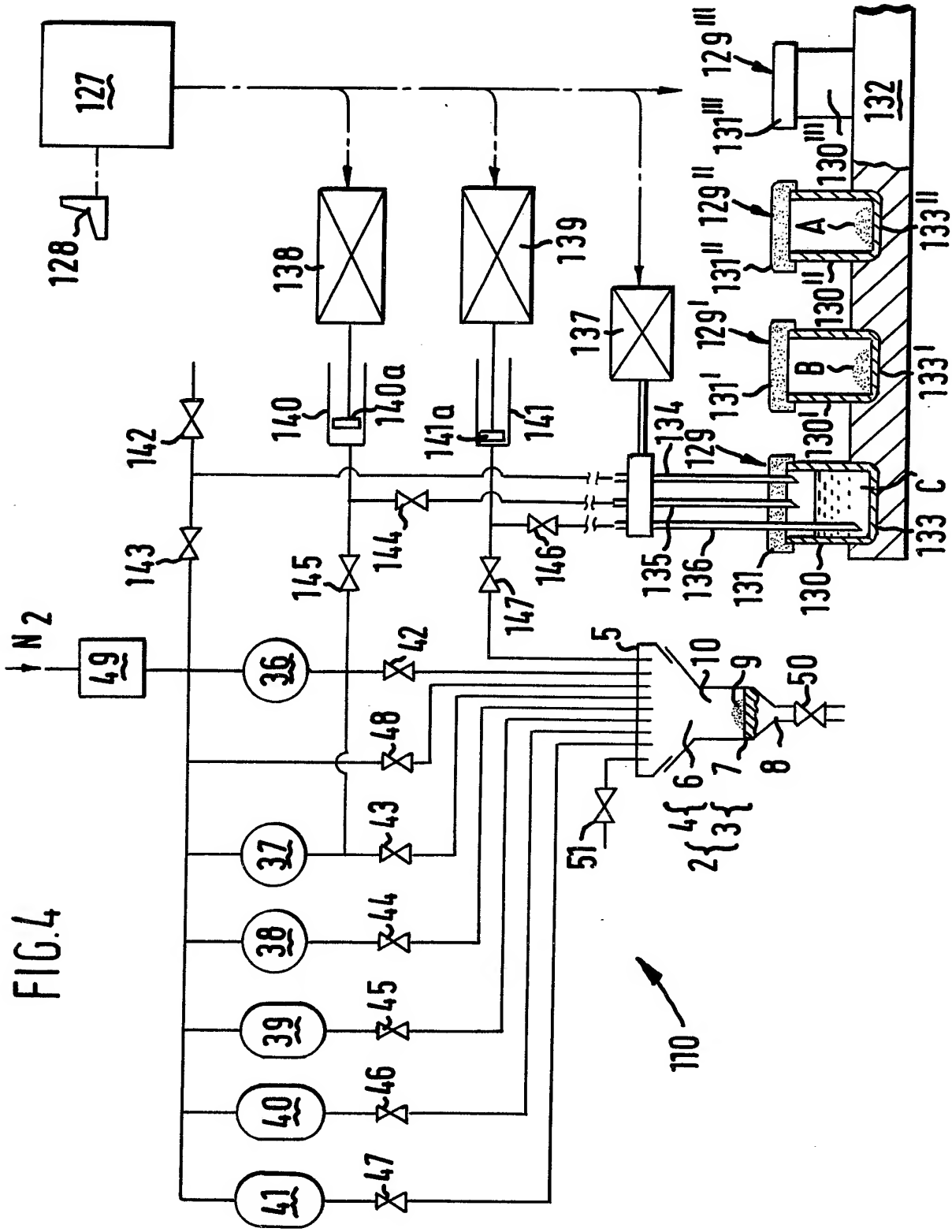
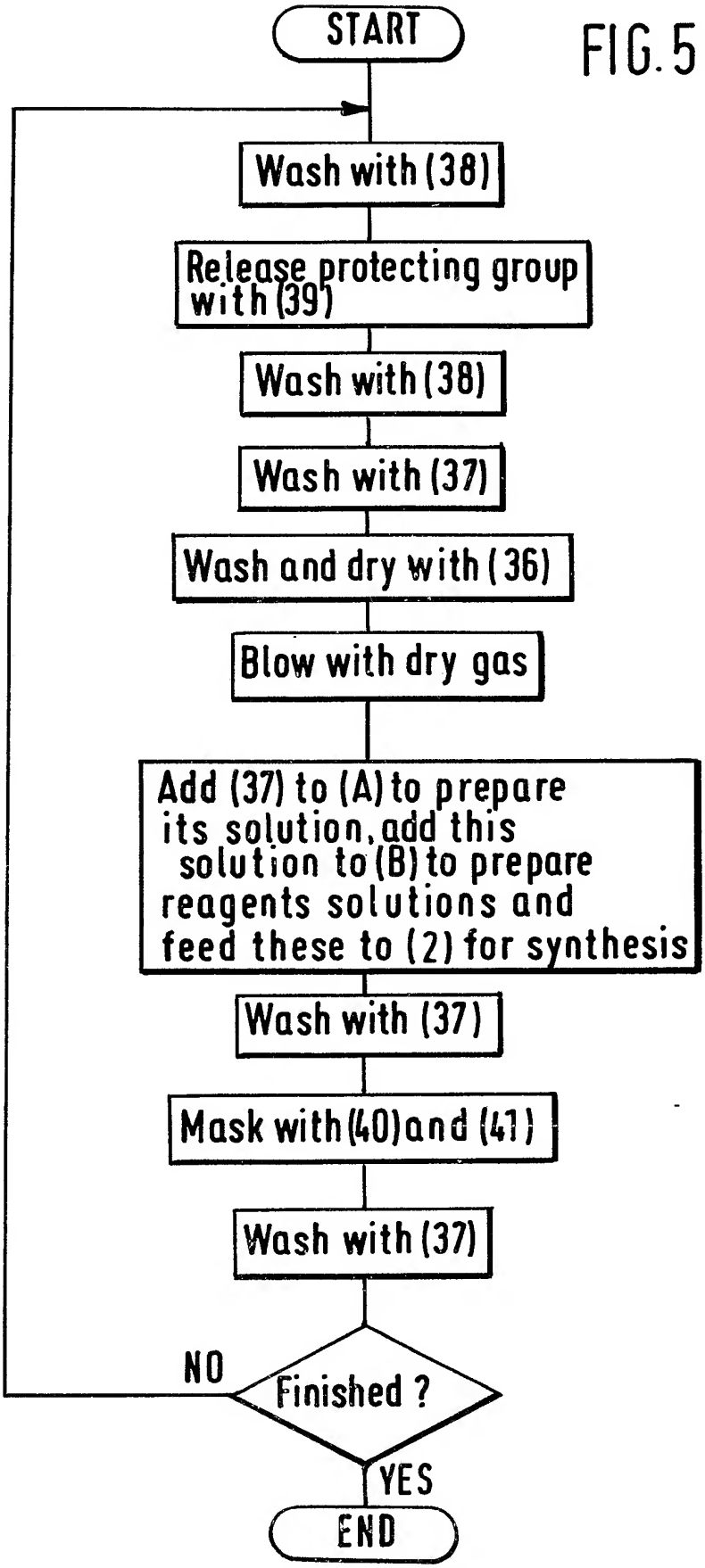

127
128
138
139
137
140
140a
141a
141
142
143
144
145
146
147
134
135
136
131
130
129
132
133 C
133'
133''
133'''
A
B
N2
49
36
37
38
39
40
41
42
43
44
45
46
47
48
5
10
9
50
8
7
6
4
3
2
51
110

FIG.4

FIG.5


START
Wash with (38)
Release protecting group with (39)
Wash with (38)
Wash with (37)
Wash and dry with (36)
Blow with dry gas
Add (37) to (A) to prepare its solution, add this solution to (B) to prepare reagents solutions and feed these to (2) for synthesis
Wash with (37)
Mask with (40) and (41)
Wash with (37)
Finished ?
NO
YES
END

SPECIFICATION

An automatic synthesizer for DNA or the like

The present invention relates to an automatic synthesizer for DNA or the like. More particularly, it relates to an instrument suited for automatic micro-synthesis of DNA or RNA.

For the synthesis of DNA improved methods have been developed to date such as the so-called diester method, triester method and phosphite method and today widely used is the solid support method by the use of a solid support, which combines the merits of the above-mentioned methods and is advantageous in many ways.

As to the reactor (reaction column) used in this solid support method, there are known three alternatives in the mode or bringing the solid support into contact with the reagent solution, i.e. the type having the reactor containing the solid support per se shaking (Polynucleotide Synthesizer by Vega Biotechnologies Inc., USA), the type having the reagent solution circulating through the reactor containing the solid support (Solid Phase Synthesizer by Genetic Design Inc., USA) and the type having a lot of reagent solution flowing once through the reactor containing the solid support (DNA/RNA Synthesizer by Bio Logicals, Canada). All these types, however, have a number of demerits as well as merits.

Meanwhile, most of the conventional automatic synthesizers were of the scales requiring more than 100 mg of solid support and wanted was development of an automatic synthesizer of a smaller scale because of the required quantity of DNA to be synthesized and the expensive nature of reagents as materials such as nucleotide having protecting group.

According to the present invention, there is provided an automatic synthesizer for DNA or the like comprising at least one reactor having an inlet for reagent solutions etc. at the top, a filter mounted at a lower level in the interior of the reactor, on which a charge of a solid support for synthesis of DNA etc. can be placed and which can pass a reagent solution, an outlet at the bottom and a reaction space of a minute volume at an upper part of said filter; a temperature control means for said reactor; a plurality of reagent solution tanks and/or containers for preparation of reagent solution; a reagent solution feed means for letting said reagent solutions out of said tanks and/or containers into said reactor; and a reagent solution feed control means for controlling said reagent solution feed means to automatically feed said plurality of reagent solutions at a definite rate or a rate according to the quantity of said support placed in said reactor in a determined order.

Main features of the invented instrument are (1) that no special means is required for effecting mixing/contact such as for shaking the reactor, (2) that is can provide a reaction system in which synthesis of nucleosides is feasible on a scale of approximately 1—5 μmol by the use of a solid support in a quantity of approximately 10—50 mg, and (3) that the required total amount of reagent solutions is only 5—7 times the volume of the solid support and, moreover, that a proper feed means provided therefor. While, the reaction space of the reactor is preferably 80 μl—800 μl.

Brief description of the drawings

Fig. 1 is a schematic diagram (showing the construction) of a preferred embodiment of the automatic synthesizer for DNA or the like of the present invention.

Fig. 2 is an illustration showing the content of the conversion table of the instrument shown in Fig. 1.

Fig. 3 is another illustation showing an example of the working display of the instrument shown in Fig. 1.

Fig. 4 is a schematic diagram (showing the construction) of another embodiment of the automatic synthesizer for DNA or the like.

Fig. 5 is a flow chart for the instrument shown in Fig. 4.

Now referring to the preferred embodiment shown in the appended drawings, the present invention is explained in detail. The invention, however, shall not be limited thereby or thereto.

In Fig. 1 shown as 1 is an automatic synthesizer for DNA according to the present invention, which works by phosphotriester method, and is basically composed of a reactor 2, a temperature control means 60, reagent solution tanks 11—15 and 36—41, a reagent solution feed means comprising changeover cocks 16—20, syringe pumps 21—25, plunger drive units 26—39 and valves 42—48 and 50—55, and a control circuit 34 functioning as a reagent solution feed control means.

The reactor 2 is a container consisting of a cylindrical part or body 3, 8 mm in inner diameter and 10 mm high, and an upper conical part of flange 4. The conical flange 4 has set in its upper rim a stopper 5 with a plurality of nozzles set therethrough for the feeding of reagent solutions. The top opening of the cylindrical body 3 serves as the inlet 6 for reagent solution etc. The cylindrical body 3 has set therein near the bottom a filter 7 such as a glass filter and an outlet 8 in its bottom. The filter 7 can have place thereon a charge of a solid support 9 such as polystyrene or silica beads (not allowing it to pass through), being permeable to the reagent solutions, solvents and gases. The space in the cylindrical body above the filter 7 constitutes a reaction space 10 whose volume is approximately 450 μl.

The temperature control means 60 is for enabling drying of the support and a reaction to proceed at a constant temperature, e.g., within a predetermined range of 20—80°C, preferably 30—40°C, consisting, for instance, of a known type of heater block.

The reagent solution tanks 11—15 and 36—41 have stored or dwelling therein respective reagent solutions or solvents. 11 is the tank for a condensing agent, which in this case is a solution

of 2,4,6-trimethylbenzene-sulfonyl-3-nitro-triazolide (MSNT) in pyridine. 12—15 are for various necleotide reagents of adenine, cytosine, guanine and thymine. 36—38 are for solvents, namely tetrahydrofuran (THF) as a volatile solvent for drying, pyridine as a solvent for washing and a mixture of isopropanol and methylene chloride as another solvent for washing. 39 is for a releasing agent for protecting group e.g., a solution zinc bromide in a mixed solvent of isopropanol and methylene chloride. 40 is for a reagent for masking, which in this case is a mixture of acetic anhydride and pyridine. 41 is for a condensing agent for masking, which is a solution of dimethylaminopyridine in pyridine.

Now described is the reagent solution feed means the syringe pumps 21—25 suck the reagent solutions 11—15 through the changeover cocks 16—20 respectively and feed them to the reactor 2 through a mixing means composed of a liquid sump 56 and a coil for mixing 57. The plungers of the syringe pumps 21—25 are driven by the plunger drive units 26—30. The plunger drive. unit 26 consists of a pulse motor 31, a threaded shaft 32 driven thereby and a nut 33 which moves on the threaded shaft as it rotates to cause a plunger 21a to reciprocate vertically. The pulse motor 31 is pulse-controlled by a control circuit 34 such as a microcomputer. The other plunger drive units 27—30, too, are of the same construction. The solvents in the tanks 36—38 and the reagent solutions in the tanks 39—41 (hereinafter called solvents 36—38 and reagent solutions 39—41) are fed to the reactor through the valves 42—47 by means of nitrogen gas pressure.

The valve 48 is for feeding nitrogen gas direct to the reactor 2 for drying by blowing its interior, thus constituting a dry gas feed means. The nitrogen gas is dried by a drying agent 49 such as calcium chloride.

As mentioned above, the control circuit 34 is for controlling the plunger drive units 26—30, also regulating the changeover cocks 16—20 and the valves 42—48 and 50—55. Thus, it is a reagent solution feed control means, at the same time being a drying control means. Through an operation console 35 the operator can input to the control circuit 34 either directly or indirectly the order in which the reagent solutions 12—15 are to be fed. By indirect inputting of the said order is meant inputting it as the order in which individual bases are linked in DNA (hereinafter called base sequence of DNA) or alternatively as the order in which individual amino acids are linked (hereinafter called amino acid sequence) as described below.

The control circuit 34 has stored in it a table 34a for converting an amino acid sequence into the corresponding base sequence of DNA, which is thus capable of converting an amino acid sequence input through the keyboard of the operation console 35 into the corresponding base sequence of DNA. The content of the table 34a is shown in Fig. 2.

In Fig. 2 letters "A", "C", "G" and "U" signify that the bases represented are "adenine", "cytosine", "guanine" and "uracil" respectively, and amino acids are represented by the abbreviations listed below.

Amino acid (AA)	*Abbreviation*
alanine	ala
arginine	arg
asparagine	asn
aspartic acid	asp
cysteine	cys
glutamine	gln
glutamic acid	glu
glycine	gly
histidine	his
isoleucine	ileu
leucine	leu
lysine	lys
methionine	met
phenylalanine	phe
proline	pro
serine	ser
threonine	thr
tryptophane	trp
tyrosine	tyr
valine	val

By the use of this table 34a a given amino acid sequence is first converted into the corresponding base sequence of mRNA, from which can be derived the corresponding base sequence of cDNA through conversion of "G"⟷"C", "A"→"T" (thymine) and "U"→"A". This cDNA base sequence is the base sequence of the probe DNA for picking out mRNA. The base sequence of the probe DNA for picking out cDNA can be derived from the base sequence of the above-mentioned probe DNA for picking out mRNA through conversion of "C"⟷"G" and "A"⟷"T".

The operation console 35 have arranged thereon an English and a numeral keyboard, through which any amino acid sequence can be input or typed in.

Now described is the way this instrument 1 works but for convenience the explanation below is given only on the (illustrated) case of synthesis of the probe DNA for picking out mRNA corresponding to an amino acid sequence of say "aspartic acid-lysine-glutamine-tyrosine". First, "1-asp, 2-lys, 3-gln, 4-tyr/mRNA/cDNA/probe (mRNA)" is to be typed in on the operation console 35.

Then the control circuit 34 refers to the table 34a and shows on the CRT of the operation console a table as illustrated in Fig. 3 to tell the operator the corresponding base sequences of mRNA, cDNA and probe DNA derived by conversion. On the displayed table the uppermost "100" represents the input amino acid sequence beginning from the NH_2 side. "101" coming next represents the corresponding base sequence of mRNA derived through conversion by the aid of the table 34a whose content is shown in Fig. 2, the 5′ end of the sequence being on the lefthand

side of the screen and the 3′ end on the right-hand side. "102" is the cDNA's base sequence derived from the above mRNA counterpart, and "103" is the base sequence of the probe DNA for picking out the aforesaid mRNA.

As seen from the table in Fig. 3, the probe DNA has two alternative base sequences for each amino acids, 16 alternatives in all. Now the operator at the operation console 35 can use the "cursor" key on the keyboard to move the cursor (104) to under each base displayed which is considered necessary to subsequently delete it by pressing the "delete" key. Thus it is possible to reduce the number of alternatives to choose from for synthesis.

The operator can know the base sequence of the DNA to be synthesized from the final display. From the displayed table in Fig. 3, for instance, he can see that synthesis started from the support to which nucleotide having cytosine as its base, and remove the stopper 5 to enter this support into the reactor 2.

Its proper quantity is 10—50 mg in total if the support is e.g., polystyrene powder. After replacing the stopper 5, the quantity of the support entered etc. is input through the operation console 35 into the control circuit 34 and then start instruction is input.

Now the control circuit 34 operates the valves 44, 48, 50, 51 for the mixed solvent 38 of isopropanol and methylene chloride to be fed to the reactor 2 for washing of the support 9. That is, the valves 44, 51 are to be opened to feed the solvent 38 and a little while after closing the same valves 44, 51 the valves 48, 50 are to be opened for draining and then after complete draining the same valves 48, 50 are to be closed. This is to be repeated several times. After this washing the valves 45, 51 are operated to feed a protecting group releasing agent 39 to the reactor 2 and the valves 48, 50 are operated after a predetermined time to drain it. The 5′ hydroxy group of the nucleotide attached to the support 9 is protected by dimethoxytrityl group (DMTr) applied in advance as protecting group but it is thereby released from the particular site.

Now the control circuit 34 again operates the valves 44, 48, 50, 51 for the mixed solvent 38 of isopropanol and methylene chloride to be fed to the reactor 2 for washing the support 9. It also operates the valves 43, 48, 50, 51 for the interior of the reactor 2 to be washed by pyridine 37.

Then the control circuit 34 opens the valves 42, 51 to feed THF 36, a volatile solvent for drying, to the reactor 2 and then a few seconds after closing the valves 42, 51, opens the valves 48, 50 for draining it and closed the same thereafter. This is repeated several times for washing and drying of the reactor 2 interior. Then the valves 48, 50, 51 are closed and the next cycle of synthesis is proceeded with.

The control circuit 34 now operates the changeover cock 20 and the plunger drive unit 30 as well as the exhaust valve 54 to feed the nucleotide reagent solution 15 to the liquid sump 56 for nucleotide has thymine as its base. It is a usual practice to select the proper nucleotide reagent solution from 12—15 according to the base of the nucleotide to be linked next and feed it to the liquid sump 56 by operating the corresponding changeover cock, plunger drive unit and exhaust valve. To be remembered is that when the nucleotide to be linked next has a plural kinds of bases, it is necessary to feed solutions of all nucleotide reagents corresponding thereto. As shown in the lowermost row 103 of the displayed table in Fig. 3, nucleotide having adenine as base and the having guanine as base are both required for the second cycle of synthesis, hence in that case solutions of both nucleotide reagents 12 and 14 are to be supplied to the liquid sump 56. When the required nucleotime reagent solutions have been supplied to the liquid sump 56, the valves 51, 52, 55 are to be operated to feed the nucleotide solution in the liquid sump to the reactor 2. Even if there are a plurality of nucleotide reagent solutions involved, they are to be fed to the reactor 2 after uniform mixing by the mixing coil 57.

Simultaneously with the feed of the above-mentioned nucleotide reagent solution the control circuit 34 operates the changeover cock 16 and plunger drive unit 26 for the solution of the condensing agent 11 to be fed to the reactor 2 by the syringe pump 21.

As to the feed rate, it is so controlled that the total quantity of the solutions of the nucleotide reagents and the condensing agents is the required minimum for wetting of the support 9. Concretely, when the support 9 is, for instance, 1 g of polystyrene powder, the required quantity of the solution of nucleotide reagent is about 3 ml and that of the condensing agent solution about 2 ml. Needless to say, it is essential to have their concentrations properly adjusted so that such minimum quantities of the solutions contain sufficient quantities of reagents.

By the above operation a new nucleotide having the desired base is linked to the nucleotide attached to the support 9 at the predetermined site. In case the solutions of a plurality of nucleotide reagents are fed as a mixture, nucleotides having different bases are bound to be coexisting in the product of synthesis. The newly linked nucleotide has its 5′-hydroxy group blocked in advance with the protecting group of DMTr.

Upon the lapse of a predetermined time the valves 50—55 are operated for the inside of the liquid sump 56, mixing coil 57 and reactor 2 to be washed clean by pyridine 37.

In case the support 9 is polystyrene powder approximately 0.1 m mol of DNA molecule are attached per g support by the end thereof and to most of it is linked new nucleotide, although a few percent thereof remains unreacted. Hence, the control circuit 34 operates to mask the unreacted reactive group as follows. So, the circuit operates the valves 46, 47, 51 so that the reagent solution for masking 40 and the condensing agent solution

for masking 41 are fed to the reactor 2. After masking the control circuit 34 operates the valves 43, 48, 50, 51 for washing the interior of the reactor 2 with pyridine 37. The sequence of operation described above completes a cycle in which a new nucleotide is linked to those attached in advance to the support 9.

The control circuit 34 repeats the synthetic cycle from the above-mentioned releasing of protecting group in which the valves 44, 48, 50, 51 are operated to feed the mixed solvent 38 of isopropanol and methylene chloride to the said masking procedure for synthesis of the target DNA.

When the DNA synthesizer 1 in the embodiment described above is used, the reagent solutions 11—15 are always fed at the minimum rates calculated on the basis of the quantity of the support and no special operation is undertaken for mixing/contact thereof unlike with conventional counterparts, this resulting in saving of the consumption of reagent with simultaneous saving of apparatuses for mixing and bringing them into contact.

This improvement is aimed at miniaturization of the reaction space 10 of the reactor 2 as well as providing a system in which the support 9 is placed on the filter 7 for the reagent solutions 11—15 fed from above to be drained through the bottom thereof. That is, when the reagent solutions are fed from above with the drain valve 50 closed, they are retained by the support 9 for imbibing thereof and thus stay in the reaction space 10 above the filter 7 not flowing down through it. Hence the whole quantity of the reagent solutions fed is allowed to participate in the reaction with no portion thereof collecting in the dead space, this enabling the desired minimization of the reagent solution feeding rate with simultaneous elimination of mixing/contact procedure.

Furthermore, the space inside the reactor 2 can be thoroughly dried in a short time for it is washed and dried with a solvent for drying e.g. THF 36 immediately before starting of the reaction for linking new nucleotides, and further by blowing with a dry gas. If a moisture which strongly retards the condensation reaction is involved in the reagent solution, it will be removed by continuous blowing of a dry gas and thus lowering the reaction efficiency is prevented. As a result, the synthetic reaction can be conducted smoothly and successfully even in an environment relatively high in humidity and saving of the reagent solutions is accomplished without their excessive feeding. For the same reason, synthesis of even trace amounts can then be accomplished without any difficulty.

By simply inputting the amino acid sequence of a peptide, it is then also possible to accomplish the automatic synthesis of the corresponding DNA i.e. the DNA required for production of the particular peptide and/or its complementary DNA. Moreover, enabled is simultaneous synthesis of even a plural kinds of DNA, this being highly advantageous when a specific gene is to be isolated from a mixture of a variety of genes for the reason stated below.

For isolating the gene relating to the production of a specific protein from a mixture of a variety of genes, there has been known a method of estimating the base sequence of the target gene from the amino acid sequence of the particular protein, synthesizing the DNA having a base sequence complementary thereto, adding it after tagging to the mixture in question for the synthesized DNA to be linked to the target gene and then picking out the target gene carrying the tag. There, however, often exist a plurality of genetic codes for a given amino acid of protein, this resulting in many possible alternatives for DNA's base sequence. Hence in such a case it is a usual practice to synthesize all possible DNAs or part thereof and use them as a mixture. In any of the conventional instruments of this kind, however, it was impossible to simultaneously synthesize a plurality of DNA sequences, and for the purpose it was necessary to either use a plural sets of this kind of instrument or synthesize different DNAs one after another in a single set of instrument, this being highly inconvenient. This inconvenience, however, can be overcome by the use of the instrument 1 of the present invention.

Numeral 110 in Fig. 4 indicates another embodiment of the present invention, in which like components are referred to by like numerals such as reactor 2, solvents 36—38 and reagents solution 39—41, which were already described above in connection with the instrument 1, hence explanation about them are here omitted.

Containers for preparation of solutions 129, 129′, 129″ ... are composed of cylindrical containers 130, 130′, 130″ .... approx. 100—500 μl in net capacity and silicone rubber septums 131, 131′, 131″ ..... The septums 131, 131′, 131″ .... are freely removable and through them needles 134, 135, 136 for introducing solvents etc. can be inserted from outside. These containers for preparation 129, 129′, ... are kept set in the holder holes 133, 133′ .... on a turn table 132 whose driving is controlled by a control circuit 127 such as a microcomputer. The container for preparation 129 brought to the predetermined position on the rotating turn table 132 has its septum penetrated by needles 134—136 by a needle lifting and lowering mechanism 137. Of the needles, 134 is for introducing and discharging nitrogen gas, 135 for introducing solvent and 136 for discharging it.

A syringe pump 140 has its plunger 140a driven by a plunger drive unit 138. Through a valve 145 it sucks in pyridine 37 and discharges it into the container for preparation 129.

The other syringe pump 141 has its plunger 141a driven by a plunger drive unit 139, sucks out the solution in the container for preparation 129 and discharges it through a valve 147 into the reactor 2, or alternatively through the valve 146 into another container for preparation. The needle lifting and lowering mechanism 137, plunger

drive units 138, 139 and valves 142—147 are controlled by the control circuit 127. The control circuit 127 also controls the valves 42—48, 50, 51. The operator converses with the control circuit 127 through the operation console 128.

For synthesis of DNA the support 9 to which end portions of DNA alone are attached is placed in advance in the reactor and the containers for preparation 129, 129′, 129″ .... are filled alternately with nucleotide reagents (A) and a condensing agent [2,4,6-trimethylbenzene-sulfonyl-3-nitro-triazolide (MSNT)] (B) both in powder form. When the support 9 is, for instance, a polystyrene powder, its proper quantity is 10—50 mg. The proper quantity of the nucleotide reagent (A) is 3—5 equiv. of the quantity of nucleotide attached to the support. So, if the support 9 is polystyrene powder and the quantity of nucleotide attached to it is 0.1 mmol/g, the proper quantity of nucleotide reagent is 400 mg/g support in the case of monomer and 700 mg/g in the case of dimer, while approximately 300 mg/g support may be proper for the condensing agent (B) in the same case. There are at least 4 kinds of nucleotide reagents (A), including 4 kinds of base monomers to be contained, and the order in which these are filled in the containers for preparation is to be determined according to the base sequence of the DNA to be synthesized. The same applies also to where the bases are dimers or trimers or mixtures thereof. Setting of the above-mentioned nucleotide reagents (A) and condensing agent (B) may be done at any time before the start of DNA synthesis.

Since the basic function of this instrument 110 is essentially the same as described above for the above-mentioned instrument 1, general explanation about it is to be omitted save for showing the flow chart in Fig. 5, and only the characteristic synthetic steps are to be described in detail.

In the synthesizing process the control circuit 127 operates the valve 145 and the plunger drive unit 138 for a small amount of pyridine 37 sucked into the syringe pump 140 and then operates the valves 144, 142 for pyridine 37 to be supplied to the container for preparation of reagent 129. The quantity of pyridine is preferably determined on the basis of the quantity of the support 9, and when, for instance, the support 9 is polystyrene powder, it is advisable to make it approx. 5 ml/g support. The nucleotide reagent (A) in the container for preparation 129 is dissolved in pyridine 37, hence after the lapse of a predetermined time the content of the container for preparation 129 is a solution of nucleotide reagent. This state is shown in Fig. 4. The control circuit 127 operates the valve 143, valve 146 and plunger drive unit 139 for the nucleotide reagent solution (C) to be sucked by the syringe pump 141. Then the needle lifting and lowering mechanism 137 is operated for the needles 134—136 to be pulled out of the container for preparation 129, the turn table 132 is driven to bring the next container for preparation 129′ to the predetermined position and the needles 134—136 are lowered into the container 129′. Then the nucleotide reagent solution (C) is supplied to the container 129′ and in it the condensing agent (B) is dissolved. After the lapse of a predetermined time the content of the container 129′ is a reagent solution containing nucleotide reagent (A) and condensing agent (B), hence the valves 143, 146, 147 are then operated to feed this reagent solution to the reactor 2. Condensation reaction is now set in the reactor 2 and new units of nucleotide are linked to the end portions of DNA attached to the support.

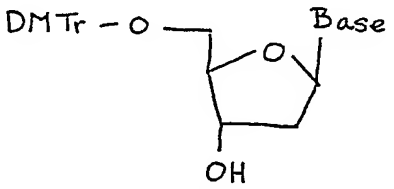
Generally, not a few of the reagent solutions used for DNA synthesis are unstable. For instance, the condensing agent solution used in the phosphotriester method, the nucleotide reagent solution used in the phosphomonotriazolide method and the nucleotide reagent solution used in the phosphite method are unstable and have to be used in a few hours after preparation. The fully anhydrous nucleotide reagent solution used in the phosphotriester method, too, has to be prepared each time before it is used. In any conventional instrument of the kind, therefore, the operator had to prepare reagent solutions and set them in the instrument each time before starting DNA synthesis, this being quite inconvenient. Sometimes in practice they were prepared and set in advance but it was always accompanied by a risk of sufficient synthesis being infeasible or difficult.

With the automatic synthesizer for DNA 110 described above, however, the nucleotide reagent (A) and the condensing agent MSNT (B) are to be stocked in the form of stable powder and it is only immediately before use that they are dissolved, i.e. made into unstable solutions. Hence with it sufficient synthesis can be achieved without fail even if the reagents etc. are set beforehand and the synthesis of DNA is started at any time, this being highly convenient. That is, with it is no longer necessary to prepare reagent solutions each time before starting DNA synthesis, this largely facilitating maintenance of the instrument. Also, it is possible to recover the residual reagents even when the reaction is stopped halfway, this ensuring against waste of the expensive reagents.

As further embodiments of the invention may be cited modifications of the above-described instruments 110 adapted to be synthesis of DNA or the like by the phosphomonotriazolide, phosphite or diester method.

Now described below is the case where the above instrument 110 is adapted to the phosphomonotriazolide method. In the arrangement shown in Fig. 4 the valve 145 is then connected to a new tank instead of the tank for pyridine 37 and this new tank is filled with a phosphating reagent such as o-chlorophenyl phosphoroditriazolide to be able to supply to the containers for preparation 129, 129′ ..... . The nucleotide derivatives of the formula (i) are filled in the containers for preparation 129, 129′, 129″ .... in

the order matching the base sequence of the DNA to be synthesized.

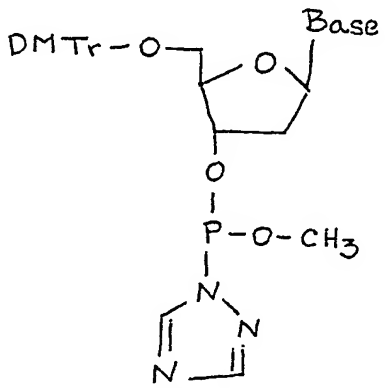


(i)

[Base is adenine, guanine, cytosine or thymine]

The syringe pump 141, plunger drive unit 139 and valve 146 may be dispensed with. Nucleotide reagent solutions are prepared by adding o-chlorophenyl phosphoroditriazolide solution to each of the nucleotide derivatives of the formula (i) and these are allowed to react for a predetermined time. These solutions of nucleotide reagents are unstable but the nucleotide derivatives of the formula (i) and o-chlorophenyl phosphoroditriazolide are stable respectively, hence the desired effect can be achieved. Likewise, described below is the case where it is adapted to the phosphite method.

The valve 145 is then connected to the tank for THF 36 instead of the tank for pyridine 37. The nucleotide derivatives of the formula (ii) are filled in the containers for preparation 129, 129′, 129″ .... in the order matching the base sequence of the DNA to be synthesized.



(ii)

[Base is adenine, guanine, cytosine or thymine]

The syringe pump 141, plunger drive unit 139 and valve 146 may be dispensed with. Nucleotide reagent solutions are prepared by adding THF to each of the nucleotide derivatives of the formula (ii).

As further embodiments of the invention may be cited those having a plurality of reactors to be substantially good for simultaneous synthesis of different kinds of DNA etc. as well as those having the reactor in the shape of a funnel or barrel or those with a reactor's net capacity of 80—800 μl. Also, cited are those in which Kel-F.g styrene, silica gel, polyacrylmorpholide etc. are used as solid support.

Such supports may preferably be in a particle size of 30—300 μm.

As still further embodiments may be cited those having as reactor's stopper a silicone rubber septum so that needles as means of filling in reagents solutions etc. can be inserted therethrough.

Since the above as well as other modifications and changes are intended to be within the scope of the present invention, the foregoing description should be construed as illustrative and not in the limiting sense, the scope of the invention being defined by the appended claims.

Claims

1. An automatic synthesizer for DNA or the like comprising at least one reactor having an inlet for reagent solutions etc. at the top, a filter mounted at a lower level in the interior of the reactor, on which a charge of a solid support for synthesis of DNA etc. can be placed and which can pass a reagent solution, an outlet at the bottom and a reaction space of a minute volume at an upper part of said filter; a temperature control means for said reactor; a plurality of reagent solution tanks and/or containers for preparation of reagent solutions; a reagent solution feed means for letting said reagent solutions out of said tanks and/or containers into said reactor; and a reagent solution feed control means for controlling said reagent solution feed means to automatically feed said plurality of reagent solutions at a definite rate for a rate according to the quantity of said support placed in said reactor in a determined order.

2. An automatic synthesizer as claimed in claim 1, wherein said minute volume of said reaction space in said reactor is 80—800 μl.

3. An automatic synthesizer as claimed in claim 1, wherein said reactor has its upper part formed as funnel-shaped flange, has a liquid outlet at the bottom, has its lower part formed as a cylindrical container with said filter set therein at a lower level and has a stopper fitted from above in said funnel-shaped flange.

4. An automatic synthesizer as claimed in claim 1, wherein said containers for preparation of reagent solutions are provided with each one removable rubber septum and can be closed tight thereby.

5. An automatic synthesizer as claimed in claim 4, wherein said reagent solution feed means is composed of a discharge nozzle, a needle, a needle shifting means for relatively shifting said needle with respect to each of said plurality of containers for preparation of reagent solutions and inserting or withdrawing it through said rubber septum thereof, and a liquid transfer means for sucking said reagent solutions prepared in said containers for preparation of reagent solutions through said needle and discharging it into said reactor through said discharge nozzle.

6. An automatic synthesizer as claimed in claim 1, wherein it further comprises an input means for inputting an amino acid sequence of peptide and a conversion means for converting said input amino acid sequence into more than one base sequence of DNA corresponding thereto, said conversion means outputting the

order of said more than one base sequence to said reagent solution feed control means as said determined order.

7. An automatic synthesizer as claimed in claim 1, wherein it further comprises a feed means for a volatile solvent for drying, a dry gas feed means and a drying control means.

8. An automatic synthesizer for DNA as claimed in claim 1, substantially as hereinbefore described and exemplified and with reference to the accompanying drawings.

9. An automatic synthesizer for DNA, whenever synthesized with the use of the automatic synthesizer according to any of claims 1 to 8.

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